BIRCH, STEWART, KOLASCH & BIRCH, LLP
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Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name, that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: USE OF COLLAGENASE 3 FOR DETECTING DESTRUCTIVE DISEASES OF THE JOINTS, ESPECIALLY FOR PROGNOSING THE PROGRESSION OF THE DISEASE AND THE GENETIC PREDISPOSITION FOR RHEUMATOID ARTHRITIS

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,
the specification was filed on _____ as
United States Application Number _____;
and amended on _____ (if applicable) and/or
the specification was filed on March 24, 2000 as PCT
International Application Number PCT/DE00/00881; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
199 13 428.6 (Number)	Germany (Country)	March 25, 1999 (Month/Day/Year Filed)	☒ Yes	☐ No
_____ (Number)	_____ (Country)	_____ (Month/Day/Year Filed)	☐ Yes	☐ No
_____ (Number)	_____ (Country)	_____ (Month/Day/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

Insert Provisional Application(s): (if any)

_____ (Application Number)	_____ (Filing Date)
_____ (Application Number)	_____ (Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Insert Requested Information: (if appropriate)

Country	Application Number	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S. Application(s): (if any)

_____ (Application Number)	_____ (Filing Date)	_____ (Status - patented, pending, abandoned)
_____ (Application Number)	_____ (Filing Date)	_____ (Status - patented, pending, abandoned)

I hereby appoint the practitioners at CUSTOMER NO. 2292 my attorneys or agents to prosecute this application and/or an international application based on this application and to transact all business in the United States Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the practitioners, unless the inventor(s) or assignee provides said practitioners with a written notice to the contrary:

Send Correspondence to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP or **CUSTOMER NO. 2292**
P.O. Box 747 • Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full Name of First or Sole Inventor: Insert Name of Inventor → Insert Date This Document is Signed

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Dirk WERNICKE	D. Wernicke	26/11/01
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Insert Residence Insert Citizenship →

Insert Post Office Address →

2-00 Full Name of Second Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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3-00 Full Name of Third Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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4-00 Full Name of Fourth Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Claudia SCHULZE WESTHOFF	Claudia Schulze Westhoff	3.12.01
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Full Name of Fifth Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Sixth Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

*DATE OF SIGNATURE

FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
3658-0103P	09/979507 NEW

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/00881	March 24, 2000	March 25, 1999

TITLE OF INVENTION
USE OF COLLAGENASE 3 FOR DETECTING DESTRUCTIVE DISEASES OF THE JOINTS, ESPECIALLY FOR PROGNOSING THE PROGRESSION OF THE DIESASE AND THE GENETIC PREDISPOSITION FOR RHEUMATOID ARTHRITIS

APPLICANT(S) FOR DO/EO/US
WERNICKE, Dirk; GROMNICA-IHLE, Erika; FREUDICTER, Dirk; SCHULZE, Claudia Westhoff

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 00/58502
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98, Form PTO-1449(s), and International Search Report (PCT/ISA/210) with 0 cited document(s).
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
    1.) Three (3) sheets of Formal Drawings
    2.) Petition to Revive

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/979507 NEW	PCT/DE00/00881	3658-0103P

21. ☒ The following fees are submitted.		CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):			
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.	**$1,040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.	**$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$ 1040.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		**$ 130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	12 - 20 =	0	X **$18.00**	**$ 0**	
Independent Claims	1 - 3 =	0	X **$84.00**	**$ 0**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		None	+ **$280.00**	**$ 0**	
		TOTAL OF ABOVE CALCULATIONS =		**$ 1170.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				**$ 0**	
		SUBTOTAL =		**$ 1170.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				**$ 0**	
		TOTAL NATIONAL FEE =		**$ 1170.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				**$ 0**	
		TOTAL FEES ENCLOSED =		**$ 1170.00**	
				Amount to be: refunded	**$**
				charged	**$**

a. ☒ A check in the amount of $ **2450.00** to cover the above fees and the petition fee is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

Date: November 23, 2001

By _____
Gerald M. Murphy, Jr., #28,977
32,181

/cqc

PATENT
3658-0103P

#3/a

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: WERNICKE, Dirk et al. Conf.:

Int'l. Appl. No.: PCT/DE00/00881

Appl. No.: New Group:

Filed: November 23, 2001 Examiner:

For: USE OF COLLAGENASE 3 FOR DETECTING DESTRUCTIVE DISEASES OF THE JOINTS, ESPECIALLY FOR PROGNOSING THE PROGRESSION OF THE DIESASE AND THE GENETIC PREDISPOSITION FOR RHEUMATOID ARTHRITIS

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

November 23, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/DE00/00881 which has an International filing date of March 24, 2000, which designated the United States of America.--

IN THE CLAIMS:

1. (Amended) A method of detecting destructive joint diseases which comprises detecting collagenase 3 as a prognostic clinical marker for the detection of destructive joint diseases.

2. (Amended) The method according to Claim 1, wherein collagenase 3 is detected for prognosis of the progression of rheumatoid arthritis (RA).

3. (Amended) The method according to Claim 1 or 2, wherein the collagenase 3 mRNA expression is determined qualitatively and quantitatively.

4. (Amended) The method according to Claim 1 or 2, wherein the collagenase 3 antigen, both as a pro-enzyme and also in an activated from, is determined qualitatively and quantitatively.

5. (Amended) The method according to Claim 1 or 2, wherein the catalytic activity of the activated collagenase 3 is detected.

6. (Amended) The method according to Claim 1 or 2, wherein the quantitative relationships between collagenase 3 and its specific or unspecific inhibitors are determined by determination of free collagenase 3 protein and of the same bound in complexes with inhibitors and compared.

7. (Amended) The method according to Claim 1 or 2, wherein the detection is done in tissues and body fluids.

8. (Amended) The method according to Claim 1 or 2, wherein synovial membrane preparations, cartilage and bone preparations or preparations of the synovial membrane/cartilage interface, obtained in synovectomies, artificial joint replacement, inter alia operative interventions, and also in biopsies are used as tissue.

9. (Amended) The method of Claim 7, wherein synovial fluid or blood are used as body fluids.

10. (Amended) The method of Claim 1, wherein collagenase 3 is used for the detection of an increased genetic predisposition for rheumatoid arthritis (RA).

11. (Amended) The method of the increase of the clinical relevance of the meaningfulness according to Claim 1 or 2, wherein not only collagenase 3, but also further markers such as HLA antigens for the detection of a more severe progression of RA or markers such as certain patterns of HLA antigens for the detection of an increased genetic predisposition are used.

12. (Amended) The method according to Claim 1 or 2, wherein

not only collagenase 3, but also MT1-MMP and/or gelatinase A act as prognostic markers by determination of their mRNA or protein expression, their amount and localization or their catalytic activity in tissues or body fluids.

Docket No. 3658-0103P

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have been amended to delete improper multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Gerald M. Murphy, Jr., #28,977

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Falls Church, VA 22040-0747
(703) 205-8000

GMM/cqc
3658-0103P

Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

Docket No. 3658-0103P

VERSION WITH MARKINGS SHOWING CHANGES MADE

The claims have been amended as follows:

1. (Amended) [Use]A method of detecting destructive joint diseases which comprises detecting collagenase 3 as a prognostic clinical marker for the detection of destructive joint diseases.

2. (Amended) [Use]The method according to Claim 1, wherein collagenase 3 is [used]detected for prognosis of the progression of rheumatoid arthritis (RA).

3. (Amended) [Use]The method according to Claim 1 or 2, wherein the collagenase 3 mRNA expression is determined qualitatively and quantitatively.

4. (Amended) [Use]The method according to Claim 1 or 2, wherein the collagenase 3 antigen, both as a pro-enzyme and also in an activated from, is determined qualitatively and quantitatively.

5. (Amended) [Use]The method according to Claim 1 or 2, wherein the catalytic activity of the activated collagenase 3 is detected.

6. (Amended) [Use]The method according to Claim 1 or 2, wherein the quantitative relationships between collagenase 3 and its specific or unspecific inhibitors[, as the case may be,] are determined by determination of free collagenase 3 protein and of the same bound in complexes with inhibitors and compared.

7. (Amended) [Use]The method according to [one of the Claims 1 to 6]Claim 1 or 2, wherein the detection is done in tissues and body fluids.

8. (Amended) [Use]The method according to Claim 1 or 2, wherein synovial membrane preparations, cartilage and bone preparations or preparations of the synovial membrane/cartilage interface, obtained in synovectomies, artificial joint replacement, inter alia operative interventions, and also in biopsies are used as tissue.

9. (Amended) [Use]The method of Claim 7, wherein synovial fluid or blood are used as body fluids.

10. (Amended) [Use]The method of Claim 1, wherein collagenase 3 is used for the detection of an increased genetic predisposition for rheumatoid arthritis (RA).

11. (Amended) [Use]The method of the increase of the clinical relevance of the meaningfulness according to [one of Claims 1 to 10]Claim 1 or 2, wherein not only collagenase 3, but

also further markers such as HLA antigens for the detection of a more severe progression of RA or markers such as certain patterns of HLA antigens for the detection of an increased genetic predisposition are used.

12. (Amended) [Use]The method according to [one of the Claims 1 to 11]Claim 1 or 2, wherein not only collagenase 3, but also MT1-MMP and/or gelatinase A act as prognostic markers by determination of their mRNA or protein expression, their amount and localization or their catalytic activity in tissues or body fluids[, as the case may be].

31PRT5

USE OF COLLAGENASE 3 FOR DETECTING DESTRUCTIVE DISEASES OF THE JOINTS, ESPECIALLY FOR PROGNOSING THE PROGRESSION OF THE DISEASE AND THE GENETIC PREDISPOSITION FOR RHEUMATOID ARTHRITIS

Description

The invention relates to the use of collagenase 3 for detecting destructive joint diseases, especially as a prognostic marker for the clinical course of rheumatoid arthritis (RA) and its genetic predisposition.

RA is a chronically inflammatory disease, predominantly of the joints. The aetiology of the disease as well as relevant pathogenetic mechanisms have remained unknown up to the present. With it being a chronic disease, patients are affected by it for years or decades. The clinical course of the disease is very heterogeneous, changing over the course of the years and decades, and cannot be forecast as yet. An early, adequate forecasting of the progression of the disease to be expected and recognition of the start of a serious progression of the disease in good time are of great importance for the patient and the doctor treating the patient, in order to treat the chronic disease, which has been incurable up to now, efficiently at least in the early stages and thus to decelerate or stop a progression as early as possible. This particularly relates to the stoppage of the process of the progredient cartilage and bone destruction.

Medicinal therapies available at present are effective, albeit frequently connected with serious side-effects. This is connected both with the effective mechanism of the medications themselves, simultaneous combined administration and the necessity of a life-long therapy for years and decades. Currently, combination therapies of

(a) steroid preparations
(b) immune suppressiva and cytostatics, so-called disease-modifying anti-rheumatic drugs (DMARD, so-called basic therapeutics) and
(c) non-steroidal anti-inflammatory medications

are in use.

On the heterogeneity and pathogenesis of RA

RA is a chronically inflammatory disease characterized by a high degree of heterogeneity, which affects the pattern of the disease, the capability of reacting to therapeutic measures (internal and surgical) and the prognosis of the disease. The clinical heterogeneity is associated with a variety of histo-pathological alterations in the synovial membrane of the joints affected. This clinical and histo-pathological heterogeneity of the disease, combined with insufficient knowledge about aetiology and relevant pathogenetic mechanisms, have led to very insufficient treatment results of RA patients up to now. Within the first two years after the on-set of the disease, about one-third of the RA patients have to give up their professional activity. In internal medicine/rheumatological departments, the share of RA patients is above 50%, in institutions of orthopaedic rheumatology even about 75%. The expenditure for long-term medicinal therapies and stationary therapeutic measures are above-average. The prevalence of the disease amounts to about 1% in the overall population.

The pathogenetic measures in the joints affected include a chronic inflammation, an abnormal immune response and a hyperplasia of the synovial membrane. The chronically inflammatory and hyperplastic synovial membrane invades into neighbouring cartilage and bone structures, thus leading to a progredient joint destruction. The clinical end point of the disease is decisively determined by the cartilage and bone destruction, which lead to a loss of function of the joints and to invalidity. However, the anti-inflammatory therapy currently in the foreground of medical therapies only has a low influence on the progression of the cartilage and bone destruction. There are indications for the fact that chronic inflammation and progredient cartilage and bone destruction are rather to be regarded as two separate pathophysiologic entities in a common pathogenetic process.

Prognostic markers available or known up to now for the progression of RA

(1) The currently safest markers used in routing in clinical practice for the progression of RA are systemic inflammation parameters such as the erythrocyte sedimentation rate, above all the C-reactive protein (CRP) and, with limitations, other acute-phase

proteins. These parameters correlate best with the current acute inflammatory activity in the organism and are the decisive parameters for an anti-inflammatory therapy. As the systemic chronic inflammation and the progredient joint destruction are only conditionally connected with one another pathogenetically, the meaningfulness of these parameters for the prognosis and the progression of the disease is very limited overall, in particular for the progredient cartilage and bone destruction.

(2) The existence of a positive rheumatoid factor is valued as an indicator for a disturbed immune response. However, it is not specific for RA and the meaningfulness for the progression of the disease is limited.

(3) It is further known that certain patterns of antigens which can be detected on the cell surface of lymphocytes and other tissue cells (so-called HLA antigens) are associated with more severe course of RA. Due to the large variety of the HLA antigens and their various epidemiological distribution, comments on the forecast of the RA are only possible in a very limited way. Correlations to the progredient joint destruction were not found.

Reliable prognostic markers of the disease are thus decisive for an early adequate medical treatment and the justification of such a therapy with regard to the side-effects also to be considered.

Therefore, the invention was based on the task of finding corresponding reliable parameters and providing corresponding markers which are particularly suited for RA.

The invention is based on the knowledge that collagenase 3 is involved in the process of progredient cartilage and bone destruction. For the progredient cartilage and bone destruction in RA, various proteases, but above all the matrix metalloproteinases (MMPs) are responsible, these being able to cleave various components of the extra-cellular matrix. Collagenase 3 as a representative of the MMP family is of particular interest for the cartilage and bone destruction in destructive joint diseases, such as RA. On the one hand, collagenase 3 possesses a high catalytic activity towards collagen type II, the main collagen component of hyaline cartilage, compared with other human collagenases and other MMP's, and cleaves a broad range of other components of the extra-cellular

matrix with high efficiency. On the other hand, it has been shown that collagenase 3 can only be detected in adult human tissues under pathological conditions, for example in the growth of malignant tumours, in chronic wounds, as well as in arthritic cartilage and in the synovial membrane in RA. It can therefore be presupposed that this MMP plays a decisive role in the progredient cartilage and bone destruction, in particular also in RA, due to the substrate specificity and the expression pattern of collagenase 3.

An increased concentration of various MMPs was detected in the synovial fluid of RA patients. However, a correlation to systemic inflammation parameters such as BSG and CRP was only shown for stromelysin 1. In addition, no correlation was detected between the collagenolytic activity in the synovial fluid and the degree of cartilage and bone destruction.

In the invention, collagenase 3 is used as a prognostic marker in destructive diseases of the joints, preferably for the detection of a progression of RA.

With regard to the chronic and changing progression of the disease, the determination of collagenase 3 is used both for prognosis in the first diagnosis and also for the control of the progression of the diseases, in order, inter alia, to recognise the inception of active phases of the disease at an early stage. More serious progressions or more active phases of RA mean both a higher inflammatory activity of the patients (measured above all with the systemic inflammation parameters BSG and CRP) as well as, in particular in this case, a quicker, i.e. more progredient cartilage and bone destruction (measured inter alia by the radiological determination of the Larsen Index, MRT measurements etc.).

Collagenase 3 is intended for this both in tissues (synovial membrane preparations, cartilage and bone preparations, preparations of the synovial membrane/cartilage interface, obtained in synovectomies, artificial joint replacements, inter alia operative interventions, as well as by biopsies) and in body fluids (synovial fluid, blood).

Preferably, the following determinations of collagenase 3 are carried out:

(a) qualitative and quantitative determination of the mRNA expression, inter alia by reverse transcription - polymerase chain reaction (RT-PCR) - analysis, Northern Blot analysis,

(b) qualitative and quantitative determination of collagenase 3 antigen (both as a proenzyme and also as an activated form), inter alia by Western Blot analysis, immunological detection methods etc.,

(c) detection of the catalytic activity of the activated collagenase 3, inter alia by zymography, the detection of specific cleaved peptides etc.,

(d) detection of a disturbed quantitative relationship between collagenase 3 and its specific (tissue-specific inhibitors of MMPs) or unspecific inhibitors (α2-macroglobulin etc.) by the determination of free collagenase 3 protein and of the same bound in complexes with inhibitors, inter alia by Western Blot analysis, immunological detection methods etc.,

(e) detection of collagenase 3 mRNA or antigen in histological preparations of the synovial membrane/cartilage border layer, inter alia by in situ hybridisation or immuno-histochemistry.

In a further embodiment of the invention, collagenase 3 is also used as a potential marker for a genetic predisposition for the disease.

Collagenase 3 can act as a single marker, but can also be evaluated in combination with other markers. Further markers can be those of which either a genetic predisposition is known or presumed or of which it is at least known that they are frequently associated with more severe progressions of the disease (such as certain patterns of HLA antigens, for example HLA-DR4 or the rheumatoid factor).

In combination with other markers, the prognostic meaningfulness both for the progression of the disease, in particular under the aspect of the progredient cartilage and bone destruction, as well as for the genetic predisposition increases or can obtain a meaningfulness which becomes relevant for clinical practice.

In addition, it has been established that collagenase 3 proenzyme is activated by MT1-MMP and/or gelatinase A. In almost all cases, an mRNA expression of these two other MMPs, membrane type-1 MMP (MT1-MMP) and gelatinase A (MMP-2) takes place at the same time as the mRNA expression of collagenase 3 in synovial membrane preparations of patients with RA. In combination with collagenase 3, MT1-MMP and gelatinase A portray prognostic markers for RA through a determination of their mRNA or protein expression, its amount and localisation or its catalytic activity in tissues or body fluids, as done for collagenase 3.

The invention is explained in more detail below on the basis of embodiments of examples.

Results

Patients

36 patients with a secured diagnosis of RA in accordance with the diagnosis criteria of the American College of Rheumatology of 1987 were included in the examinations. The patients were examined clinically and para-clinically. In all patients, the wrist joints were affected by the disease. In the patients included in the examinations, a rheumatic surgery intervention for the removal of the inflammatory and hyperplastic synovial membrane (so-called synovectomy) in one of the wrist joints in each case was necessary, in order to retard a progression of the joint destruction and to improve the movement capability of the joint. The material removed surgically was both analysed histo-pathologically and also used for the preparation of mRNA. The patients were attended to with regard to internal treatment in the Clinic of Rheumatology in Berlin-Buch and operated in the Orthopaedic Department of the Berlin-Buch Hospital.

mRNA expression of collagenase 3 in the synovial membrane preparations

The mRNA expression of collagenase 3 was examined in the synovial membrane preparations of all 36 patients by Northern Blot analysis (Fig. 1). 21 preparations (60%) showed an mRNA expression of collagenase 3. As opposed to this, it is known that the mRNA expression of other MMPs, such as interstitial collagenase and stromelysin 1 is detectable in all synovial membrane preparations (in Fig. 1, only shown for interstitial

collagenase). The results of the Northern Blot analysis were confirmed by the examinations with the method of the RT-PCR. It was further found that an mRNA expression of collagenase 3 in synovial membrane preparations of patients with RA is associated in almost all cases with an mRNA co-expression of two other MMPs, membrane type-1 MMP (MT1-MMP) and gelatinase A (MMP-2). If an mRNA expression of MT1-MMP and gelatinase A was detected in the absence of a collagenase 3 mRNA expression, its expression level was distinctly lower than in a co-expression with collagenase 3 mRNA in the majority of cases. These results were received by Northern Blot analysis and with the method of the RT-PCR (results not shown here).

Figure 1 shows a representative Northern Blot with synovial membrane preparations from 6 patients with RA. 25 µl total RNA were loaded in each case. Unlike interstitial collagenase, which is expressed in all patients, an mRNA expression of collagenase 3 can only be detected in some of the RA patients. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is used as a control for the application of equivalent amounts of RNA.

Clinical and para-clinical parameters

From Table 1, it can be seen that patients with collagenase 3 mRNA expression in the synovial membrane manifest significantly increased systemic BSG ($p<0.05$) and CRP ($p<0.005$) inflammation markers. There are no differences between the two groups of patients with regard to the rheumatoid factor and in the differential haemogram.

The degree of cartilage and bone destruction was determined radiologically by means of the Larsen Index by X-rays of the hand, wrist and foot being examined. Although, on the one hand, no significant difference in the degree of bone and cartilage destruction was detected between the two groups of patients with a view to the small cohorte of patients, it was, on the other hand, conspicuous that 24% of the patient group with collagenase 3 mRNA expression in synovial membrane 5 had a time course of the disease of less than 2 years, whereas only one patient (7%) in the group without collagenase 3 mRNA expression had been suffering for less than 2 years. In addition, 6 patients (29%) in the group of patients with collagenase 3 mRNA expression had prior rheumatic surgery interventions in contrast to only 2 patients (14%) in the group of patients without collagenase 3 mRNA expression.

Thus, patients with collagenase 3 mRNA expression in the synovial membrane manifest a higher systemic inflammatory activity and are subjected to rheumatic surgery interventions at a earlier stage (the latter is connected with a more severe progression of the disease and/or with a lower response to medicinal therapies - the second begin discussed in the next point) than patients without collagenase 3 mRNA expression.

Tab. 1 Clinical and para-clinical parameters of RA patients in relation to collagenase 3 mRNA expression in the synovial membrane

Parameter	Collagenase 3 mRNA expression		Statistical
	without	with	analysis
Age,years	59.6 ± 5.6 (from 32 to 81)	58.6 ± 4.9 (from 28 to 83)	n.s.
Sex (m/f),years	3/12	3/18	n.s.
Length of disease,years	11.3 ± 3.1	10.9 ± 1.8	n.s.
Haemoglobin,mg/dl	8.3 ± 0.4	7.8 ± 0.3	n.s.
Leukocyte count	9.3 ± 1.1	10.3 ± 0.7	n.s.
BSG (mm/h)	27.0 ± 5.0	39.0 ± 5.4	$p < 0.05$
CRP (mg/l)	9.2 ± 2.7	30.9 ± 6.0	P y 0.005
Rheumatoid factor positive/ Number of patients (%)	9/15 (60%)	14/21 (66%)	n.s.

Medicinal therapy

In accordance with the higher systemic inflammation activity in patients with collagenase 3 mRNA expression in the synovial membrane, the latter was treated more frequently with Prednisolon (Fig. 2A). In addition, 15 patients (71%) with collagenase mRNA 3 expression in the synovial membrane were receiving at least 5 mg/ml Prednisolon as compared with 7 patients (47%) without collagenase 3 mRNA expression at the time of the synovectomy. Therapy with DMARD was carried out as single drug therapy.

DMARD were prescribed and changed in the following order (matching the aggressiveness of their effect): Chloroquin (250 mg/d), Sulfasalazin (2 g/d), Methotrexat (15-20 mg/week), gold sodium thiomalate (50 mg/week) and Azathioprin (2 mg/kg/d). As shown in Fig. 2A, patients with collagenase 3 mRNA expression in the synovial membrane were treated more frequently with DMARD. In addition, the DMARD had to be changed more frequently due to a lack of efficacy in patients with collagenase 3 mRNA expression in the synovial membrane (Fig. 2B). In both groups of patients, the DMARD had to be changed for two patients per group due to side-effects.

Thus, patients with collagenase 3 mRNA expression in the synovial membrane were treated more aggressively with medications and were more resistant to an effective medical treatment. The latter possibly also led to an earlier necessity of a rheumatism-surgery intervention.

Rheumatological family anamnesis with regard to RA

In the collagenase 3 positive group of patients, 10 of 19 patients (48%) manifested a positive family anamnesis. In the patient group without collagenase 3 mRNA expression in the synovial membrane, on the other hand, members of the family of only three of 14 cases (20%) suffered from RA (Fig. 3). No information was obtained from a total of three patients. Thus, the accumulation of a positive family anamnesis in the patient group with a collagenase 3 mRNA expression in the synovial membrane is conspicuous.

Patent Claims

1. Use of collagenase 3 as a prognostic clinical marker for the detection of destructive joint diseases.

2. Use according to Claim 1, wherein collagenase 3 is used for prognosis of the progression of rheumatoid arthritis (RA).

3. Use according to Claim 1 or 2, wherein the collagenase 3 mRNA expression is determined qualitatively and quantitatively.

4. Use according to Claim 1 or 2, wherein the collagenase 3 antigen, both as a pro-enzyme and also in an activated form, is determined qualitatively and quantitatively.

5. Use according to Claim 1 or 2, wherein the catalytic activity of the activated collagenase 3 is detected.

6. Use according to Claim 1 or 2, wherein the quantitative relationships between collagenase 3 and its specific or unspecific inhibitors, as the case may be, are determined by determination of free collagenase 3 protein and of the same bound in complexes with inhibitors and compared.

7. Use according to one of the Claims 1 to 6, wherein the detection is done in tissues and body fluids.

8. Use according to Claim 7, wherein synovial membrane preparations, cartilage and bone preparations or preparations of the synovial membrane/cartilage interface, obtained in synovectomies, artificial joint replacement, inter alia operative interventions, and also in biopsies are used as tissue.

9. Use according to Claim 7, wherein synovial fluid or blood are used as body fluids.

10. Use according to Claim 1, wherein collagenase 3 is used for the detection of an increased genetic predisposition for rheumatoid arthritis (RA).

11. Use of the increase of the clinical relevance of the meaningfulness according to one of Claims 1 to 10, wherein not only collagenase 3, but also further markers such as HLA antigens for the detection of a more severe progression of RA or markers such as certain patterns of HLA antigens for the detection of an increased genetic predisposition are used.

12. Use according to one of the Claims 1 to 11, wherein not only collagenase 3, but also MT1-MMP and/or gelatinase A act as prognostic markers by determination of their mRNA or protein expression, their amount and localisation or their catalytic activity in tissues or body fluids, as the case may be.

ABSTRACT OF THE DISCLOSURE

The invention relates to the use of collagenase 3 for detecting destructive diseases of the joints, especially for prognosing the progression of the disease and the genetic predisposition for rheumatoid arthritis.

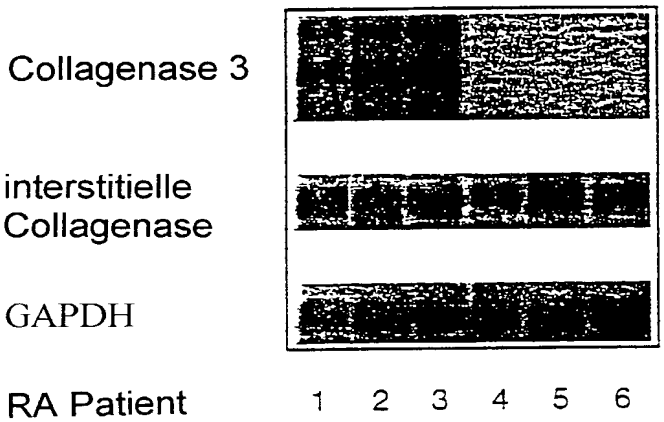
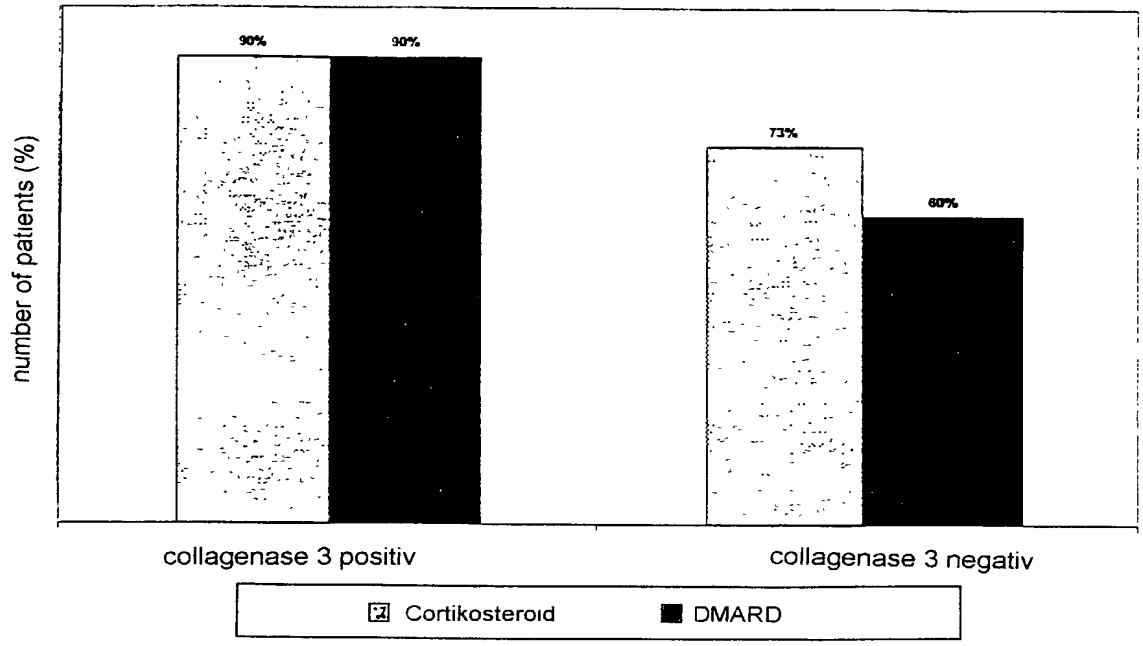


Fig.1 Northern Blot-Analysis of Collagenase 3 in preparates of synovialmembranes of patients with rheumatoid arthritis

A



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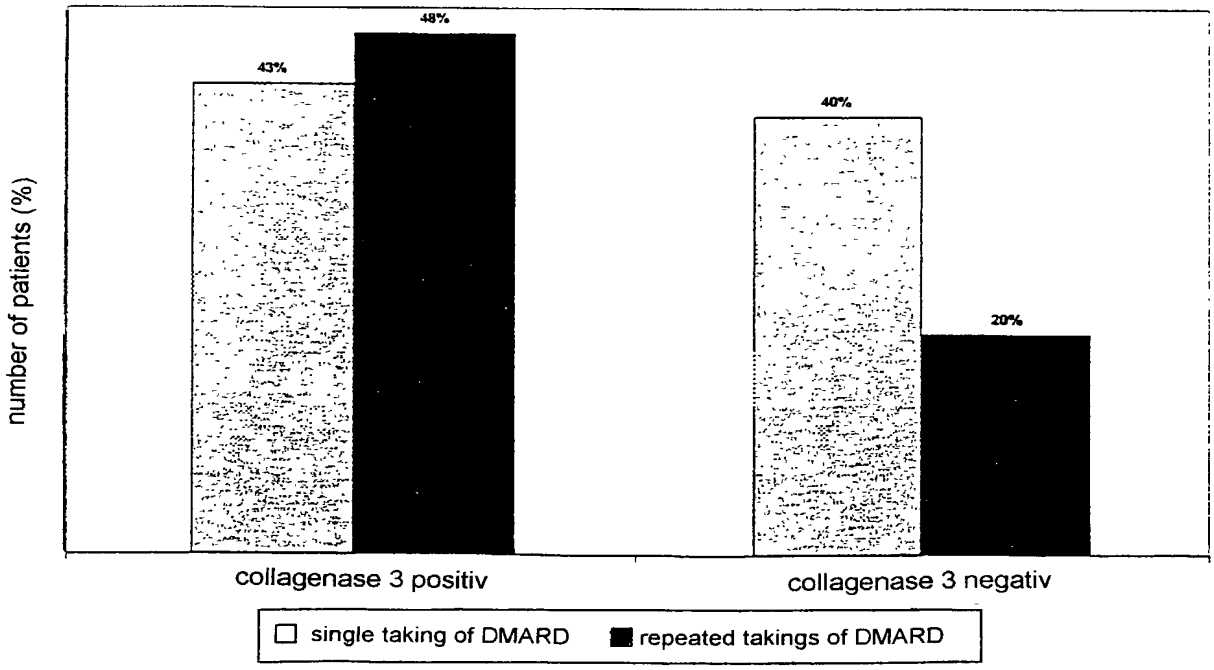

Fig. 2 Therapie with Medicaments

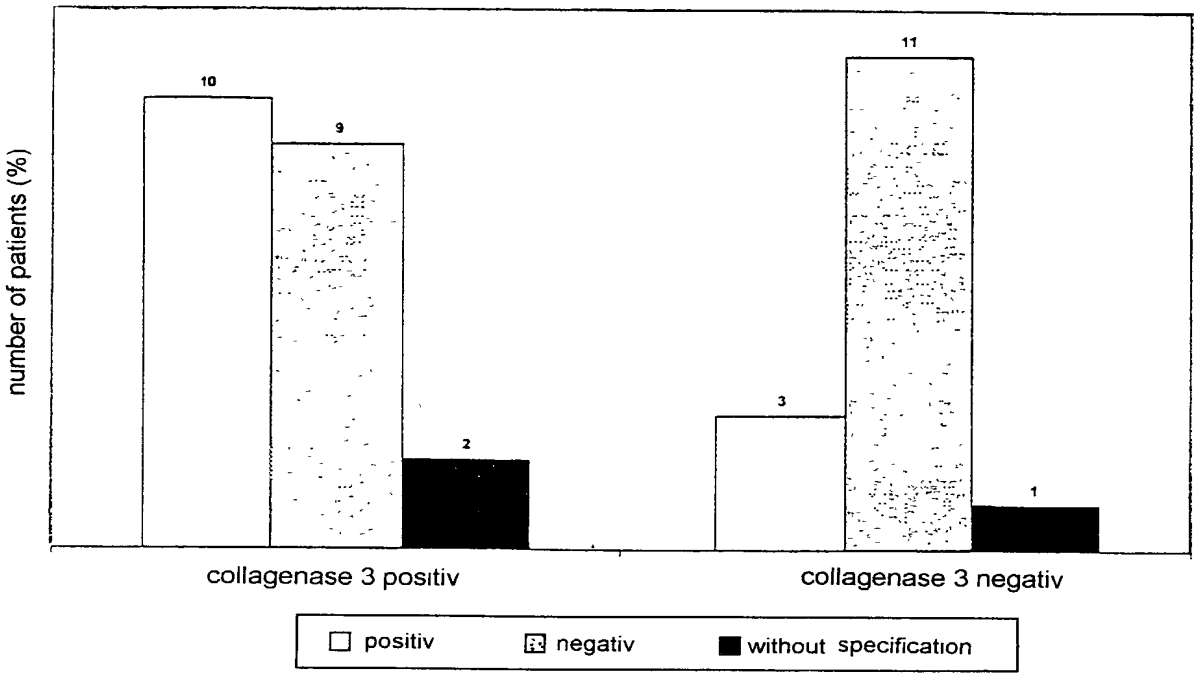

Fig. 3 Anamnesis of families refer to RA in dependence of Collagenase 3 mRNA Expression in the Synovialmembrane